

DATA EVALUATION REPORT**THIOPHANATE-METHYL****STUDY TYPE: CHRONIC ORAL TOXICITY FEEDING - RAT (83-1a)**


Prepared for

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U.S. Environmental Protection Agency
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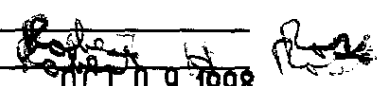
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THIOPHANATE-METHYL

Chronic Toxicity Oral Study (83-1a)

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DATA EVALUATION RECORDSTUDY TYPE: Chronic Oral Toxicity Feeding-Rat

OPPTS 870.4100 [§83-1a]

DP.BARCODE: D241367SUBMISSION CODE: S511876P.C. CODE: 102001TOX. CHEM. NO.: 375ATEST MATERIAL (PURITY): Thiophanate-methyl (>94% a.i.)SYNONYMS: Dimethyl 4,4'-o-phenylene bis(3-thioallophanate)

CITATION: Noguchi, T., Hashimoto, Y., Makita, T. *et al.* (1971) The results of intermediate data about the chronic oral toxicity studies of thiophanate-methyl in rats. III. Intermediate report after 12 months. Nisso Institute for Life Science, Nippon Soda Co., Ltd. Oiso-machi, Kanagawa-ken, Japan and Kanazawa University Dept. Of Pathology, Japan. Received on 9/5/72 under 2G1249; submitted by Pennwalt Corp., Philadelphia, PA, CDL:091777-F. MRID 00057651. Unpublished.

Taniguchi, T., Hashimoto, Y., Tsubura, Y. *et al.* (1972) Final report on the chronic oral toxicity studies of thiophanate-methyl, dimethyl 4,4'-o-phenylenebis (3-thioallophanate), in rats of Sprague Dawley strain for 24 months. Nisso Institute for Life Science, Nippon Soda Co., Ltd. Oiso, Kanagawa, Japan, Kanazawa University Dept. Of Pathology, Japan and Department of Pathology, Nara Medical University, Nara-ken, Japan. Received 9/7/72 under 2G1249, submitted by Pennwalt Corp., Takoma, WA, CDL:091779-C. MRID 00117868. Unpublished.

SPONSOR: Elf Atochem (Originally submitted by Pennwalt Corp.)

EXECUTIVE SUMMARY: In a chronic toxicity study (MRID 00117868), Thiophanate-methyl (stated purity >94% a.i.) was administered for 24 months to 40 Sprague-Dawley rats/sex/dose at 0 ppm and 25 rats/sex/dose in the diet at dose levels of 10, 40, 160, or 640 ppm (total compound intakes estimated by author as 0, 193, 769, 2813, and 11749 mg/rat for males and 0, 149, 588, 2465, and 8642 mg/rat for females, respectively; average daily doses estimated by reviewer as 0, 0.370, 1.54, 5.75, or 24.3 mg/kg/d for males and 0, 0.399, 1.62, 7.18, or 28.7 mg/kg/d for females). In an interim portion (MRID 00057651) of that same study, Thiophanate-methyl was administered in the diet to an additional 5 Sprague-Dawley rats/sex/dose for 3 months and 5 rats/sex/dose for 12 months at dose levels of 0, 10, 40, 160, or 640 ppm (estimated by reviewer as 12-month means of 0.451, 1.85, 6.79, or 29.2 mg/kg/d for males and as 0.454, 1.98, 8.52, or 35.1 mg/kg/d for females, respectively).

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At 640 ppm, slightly decreased body weight/weight gain were observed in males and females (at termination, 13%/16% and 16%/19% less than controls, respectively); weights began to decrease during the first year in females and the second year in males. In males, increased microscopic findings of increased thyroid epithelial cell columnar height and increased colloidal substance, or hypertrophy (17% vs. 2%, controls-all animals on study) and decreased spermatogenesis (17% vs. 2%, controls-all animals on study; for main study animals 24% vs. 2.5%, controls) were observed at termination. Although the incidence of grossly visible testicular atrophy was higher at 160 and 640 ppm (17% and 20%, respectively vs. 2.5%, controls), it was not used to establish a LOAEL because of the lack of correlation with microscopic lesions and the old age of the animals. No treatment-related, toxicologically significant changes were seen on mortality, clinical signs of toxicity, ophthalmological findings, cumulative food consumption (marginal decrease of about 10%, females), food efficiency marginal decreases of about 10%, both sexes), hematological, clinical chemistry or urinalysis parameters, or in absolute organ weights or organ-to-body weight percentages. **The LOAEL is 640 ppm (24.3 mg/kg/day), based on decreased body weight and weight gain in both sexes and increased incidence of thyroid and testicular microscopic effects in males. The NOAEL is 150 ppm (5.75 mg/kg/day).**

There were no treatment-related increases in neoplastic lesions observed in this study. Dosing was considered adequate based on decreased body weights and microscopic lesions in males and decreased body weights in females. However, the small number of surviving animals make conclusions as to carcinogenicity tentative.

This chronic toxicity study is classified as **Unacceptable (§83-1a)** because chemical analyses were not reported for purity or stability, homogeneity and concentration of the test material in the diet (making identification of the LOAEL/NOAEL tentative) and required individual rat data are not presented for several endpoints. However, no additional data are required to upgrade this study at this time because an acceptable chronic toxicity/carcinogenicity study in the rat has been submitted for Thiophanate-methyl (MRID 42896601; see review in HED Doc. No. 011531).

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality and Flagging statements were not provided. These studies were conducted prior to publication of current guidelines for GLP or for chronic toxicity studies.

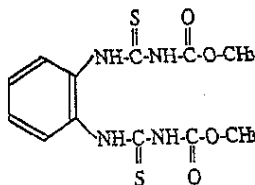
I. MATERIALS AND METHODS**A. MATERIALS****1. Test material: Thiophanate-methyl**

Description: pale to yellowish brown solid
 Lot/Batch #: not given
 Purity: > 94% active ingredient
 Stability of compound: not given
 CAS #: 23564-05-8

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Structure:

2. Vehicle and/or positive control

None, as material was administered by dietary feeding.

3. Test animals

Species: rat

Strain: Sprague-Dawley, SPF

Age and weight at study initiation: "Weanlings," age not specified; male, 115-200 g; female, 50-175 g (range of individual values)

Source: Central Laboratory of Experimental Animals

Housing: Animals were housed in standard-bottomed, stainless steel rat cages in pairs; they were isolated if showing severe debility or intoxication

Diet: Animals were fed unspecified diet *ad libitum*

Water: Drinking water was provided presumably *ad libitum* (not stated in report)

Environmental conditions:

Temperature: 23 ± 1 EC

Humidity: $55 \pm 5\%$

Air changes: Not specified

Photoperiod: Not specified

Acclimation period: Not specified; there may have been none as 2 females in 10 ppm dose group were very small, ill, and were sacrificed in first week of study

B. STUDY DESIGN1. In life dates

Not given.

2. Animal assignment

Animals were assigned to treatment groups (Table 1) by an unspecified method; initial group mean weights differed considerably (by as much as 22%).

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TABLE 1. Study design									
Test group	Doses			Number of animals					
	Dose in diet (ppm)	Dosage achieved* (mean) (mg/kg/day)		Main study (103 weeks)		Interim sacrifice (14 weeks)		Interim sacrifice (58 weeks)	
		Male	Female	Male	Female	Male	Female	Male	Female
Group-1	0	0	0	40	40	5	5	5	5
Group-2	10	0.370	0.399	25	25	5	5	5	5
Group-3	40	1.54	1.62	25	25	5	5	5	5
Group-4	160	5.75	7.18	25	25	5	5	5	5
Group-5	640	24.3	28.7	25	25	5	5	5	5

For animals sacrificed at 103 weeks, data taken or calculated from pp. 16 and Tables 4-1, 4-2, 5-1, and 5-2, pp. 55-58, MRID 00117868. For interim sacrifice animals, data taken from page 3, Table 1, p. 18, and Table 2, p. 19, MRID 00057651.

*Mean time-weighted dose as mg/kg/d during 24 months' exposure, calculated by reviewer from mean intake/rat/day estimated by study author and group mean body weight data.

3. Dose selection rationale

The dose selection rationale was not specified.

4. Diet preparation and analysis

No description of the mixing procedure is given.

Results –

Homogeneity analysis: no data are available on homogeneity.

Stability analysis: no data are available on stability.

Concentration analysis: no data are available on concentration verification at various dose levels.

Data are not available to assure that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics

No information is given on the statistical analyses used in this study. The results of the analysis on overall body weight gains are suggestive of using a paired t-test. Statistical analysis of body weight and body weight gain data was performed by the reviewer using one-way analysis of variance (ANOVA).

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C. METHODS1. Observations

Animals were observed once daily for mortality and abnormal behavior. Any animals showing severe debility or intoxication were isolated. Blood and urine samples were taken if possible, from animals *in extremis* and the animals were necropsied.

2. Body weight

Individual body weights were recorded initially, and weekly thereafter.

3. Food consumption and compound intake

Cage totals for food consumption data were recorded every 10 days during treatment.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were performed on all animals at 0, 3, 6, 12, 18, and 24 months (p. 4 of MRID 00057651).

5. Blood was collected for hematology from 5 rats/sex/dose after 3, 6, 12, 18, and 24 months of treatment; it was collected for clinical chemistry determinations at 3 and 12 months on 5 rats/sex/dose, and at 24 months on all remaining rats. Whether animals were fasted prior to blood sampling was not reported. The interim report stated that clinical chemistry samples were taken by heart puncture; the same might be assumed of hematology samples but the report did not state so, and type of anesthesia was not indicated. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*		Mean corpusc. volume (MCV)
	Platelet count*		Reticulocyte count (smear taken but not read)
	Blood clotting measurements*		Blood cell morphology
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time) (at scheduled sacrifice only)		

*Required for chronic studies based on Subdivision F Guidelines

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dose groups were examined microscopically, but it appears that aorta, bone, and skeletal muscle were not. Data sheets for individual animals indicate that "subcutaneous" and blood were also examined microscopically. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
X	Tongue (oral cavity)	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*		Periph. nerve*
X	Esophagus*	X	Bone marrow*	XX	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes (mesenteric)*		Pituitary*
X	Duodenum* ¹	XX	Spleen*		Eyes (optic n.)*
X	Jejunum* ¹	XX	Thymus* ²		
X	Ileum* ¹				
X	Cecum*			X	GLANDULAR
X	Colon* ¹	XX	UROGENITAL		Adrenal gland*
	Rectum*	X	Kidneys*+	X	Lacrimal gland
XX	Liver*+	XX	Urinary Bladder*	X	Mammary gland*
	Gall bladder*		Testes*+	XX	Parathyroids*
X	Pancreas*	X	Epididymides		Thyroids*
			Prostate		
	RESPIRATORY	XX	Seminal vesicle	X	OTHER
	Trachea*	X	Ovaries*+		Bone*
XX	Lung*		Uterus* ³		Skeletal muscle *
	Nose			?	Skin*
	Pharynx				All gross lesions and masses* ⁴
	Larynx				

* Required for chronic studies based on Subdivision F Guidelines.

+ Organ weight required in chronic studies.

¹Small intestine is listed only as that for 12-month interim sacrifice animals; nowhere is it made clear whether duodenum, jejunum and ileum were all examined. Likewise, only large intestine is listed without specifying the region examined. At 24-month sacrifice, the three regions of the small intestine were examined.

²Thymus weights were recorded and presented for animals sacrificed at 12 months (MRID 00057651); it is stated that the thymus was weighed at terminal sacrifice (24 months), but data are not presented (MRID 00117868).

³Uterine weights are said to have been recorded at 12 months interim sacrifice (MRID 00057651), but are not presented. Uterine weights are neither listed as having been obtained nor are they presented for animals sacrificed at 24 months.

⁴Some gross lesions clearly were examined histopathologically as photographs are presented and discussion suggests that many were, but it is not explicitly stated whether all were so evaluated. Some organs or tissues not listed in Material and Methods (p. 7) are listed in Tables 39-1 to 39-4, pp. 61-68, where observations were scored, MRID 00057651; likewise in MRID 00117868, some organs or tissues not listed on p. 18 are presented in Tables 16-1 to 16-40, pp. 127-166.

II. RESULTS

A. OBSERVATIONS

1. Clinical signs of toxicity

There were no test material-related antemortem observations during this study.

2. Mortality

No treatment-related effect on mortality was seen in either sex of rats in either the interim groups or the 24-month treatment groups. The mortality rates at the end

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of the two-year treatment period were, in males: 57.5%, 68%, 64%, 60%, and 68%; in females, they were: 50%, 72%, 52%, 44%, and 40% at doses of 0, 10, 40, 160, and 640 ppm, respectively. No notable differences in the age at death of animals dying prior to sacrifice were observed except for two 10 ppm females that died in the first week of treatment. Their body weights were extremely low relative to the rest of the group (50 g and 55 g compared to 140 g mean for group), suggesting that they were unhealthy prior to the onset of dosing, and they had lung lesions ("yellowish-white areas in lungs") at necropsy. Thus the increased mortality rate in that group is likely not treatment-related.

B. BODY WEIGHT AND WEIGHT GAIN

Selected mean body weight and weight gain data are presented below in Tables 2 (males) and 3 (females). Body weights appear to have been analyzed on all surviving animals at a given time point; however, no individual body weight data are presented for the animals sacrificed at 3, 12, or 24 months other than initial and final weights. Body weights were modestly lower in the 640 ppm males from months 12-24 (12 to 13% lower; 13% at termination, calculated by reviewer). Body weights were moderately lower in the 640 ppm females in months 6 to 24 during treatment (14 to 24%; 16% at termination). Terminal body weights at 24 months were evaluated by one-way analysis of variance (ANOVA) by the reviewer and no statistical differences were seen between treatment groups and controls in either male or female rats. The lack of individual data or group mean standard errors at intermediate times precludes any further statistical analysis.

Mean overall body weight gains were reported by the study author to be significantly lower than control values ($p < 0.05$) for the 640-ppm males (16%) and females (19%). However, the statistical test used appears to have been a paired t-test which is inappropriate; one-way ANOVA (performed by the reviewer) showed no statistically significant differences between any groups and controls in overall body weight gain for either male or female rats. Again, lack of individual data precludes further statistical analysis.

Although the decreases in body weight and weight gain observed at 640 ppm were not statistically significant upon reevaluation, the decreases are of a sufficient magnitude ($>10\%$ relative to controls) to be considered treatment-related.

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TABLE 2: Mean body weights (g) and body weight gains (g) of male rats administered Thiophanate-methyl in the diet for 24 months					
Month of study	Dose (ppm)				
	0	10	40	160	640
0	148	162	177	145	152
3	545	553	579	543	548
6	630	659	634	640	643
12	754	779	719	701	735 ¹
18	819	779	758	766	717 (12) ²
24	774	769	707	701 (9.4)	673 (13)
Body weight gains					
0-12	606	617	542	556 (9.0)	583 (4.0)
0-24 \pm SE	630 \pm 26	612 \pm 83	532 \pm 58	556 \pm 31 (12)	532 \pm 23 (16)

Data taken from Tables 4-1 and 4-2, pp. 55-56, MRID 00117868.

BW: body weight; SE: standard error.

¹Poor quality original prevents reading number with certainty; it is either 735 or 755.

²Numbers in parentheses are the percent decrease relative to untreated controls, calculated by the reviewer.

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TABLE 3: Mean body weights (g) and body weight gains (g) of female rats administered Thiophanate-methyl in the diet for 24 months					
Month of study	Dose (ppm)				
	0	10	40	160	640
0	120	142	146	125	122
3	344	384	367	338	330 (4.1) ¹
6	422	411	425	401	362 (14)
9	456	458	454	448	392 (14)
12	520	529	522	489	427 (18)
18	607	541	553	530	460 (24)
24	560	615	591	564	472 (16)
Body weight gain					
0-12	400	387	376	364	305 (24)
0-24 ± SE	438 ± 28	477 ± 36	450 ± 37	439 ± 35	356 ± 21 (19)

Data taken from Tables 4-1 and 4-2, pp. 55-56, MRID 00117868.

BW: body weight; SE: standard error.

¹Numbers in parentheses are the percent decrease relative to untreated controls, calculated by the reviewer.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Since animals were caged in pairs, food consumption was monitored by cage for ten-day periods and consumption per rat was obtained by dividing total consumption by two. The study author presents data only for group mean cumulative total consumption (g) at 3, 6, 12, 18, and 24 months rather than data in g/kg/day. The high-dose males consumed slightly less food overall than controls (4.8% less at 24 months). The 640-ppm females had lower cumulative consumption than controls at 12 (8.6%), 18 (12%), and 24 months (10%). The lower consumption in high-dose females is suggestive of a possible palatability problem. No consistent or dose-related decreases in food consumption were observed in other treatment groups. No individual animal or cage data are presented for food consumption in either the interim report (MRID 00057651) or the final report (MRID 00117868).

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2. Compound consumption

The study authors reported total cumulative compound intake and average daily intakes (mg/animal/day) at 3, 6, 12, 18, and 24 months. Total cumulative compound intake at 24 months (control to high dose, respectively) was 0, 193, 769, 2813 and 11749 mg/animal, males and 0, 149, 588, 2465 and 8642 mg/animal, females. As of 12 months' exposure, the estimated average daily time-weighted doses for males calculated by the reviewer at 0, 10, 40, 160, and 640 ppm are 0, 0.432, 1.85, 6.90, and 29.3 mg/kg/day, respectively, and for females, the estimated average daily doses are 0, 0.490, 1.98, 8.52, and 36.2 mg/kg/day, respectively. For the overall 24-month study, the reviewer calculated average daily time-weighted doses for males as 0.370, 1.54, 5.75, and 24.3 mg/kg/day and for females as 0.399, 1.62, 7.18, and 28.7 mg/kg/d for the 10, 40, 160, and 640 ppm dose groups, respectively (Table 1). The compound consumption calculations were based on the nominal concentrations of test substance in the diet, as no results of concentration analysis were reported on which to base the intake and dose estimates, and the group mean body weights.

3. Food efficiency

No food efficiency calculations were performed by the study author. The reviewer calculated overall food efficiency values as of 24 months to be 0.03266, 0.03175, 0.02768, 0.03162, and 0.02898 cumulative g weight gain/cumulative g food consumed for male rats receiving 0, 10, 40, 160, and 640 ppm Thiophanate-methyl, respectively. No dose response is evident in the male groups. Values for corresponding female dose groups were 0.02909, 0.03210, 0.03060, 0.02850, and 0.02636 g/g, respectively. While the high-dose group means were slightly lower than control values (by 11% for males and 9.4% for females), these differences are of uncertain toxicological significance.

D. OPHTHALMOSCOPIC EXAMINATION

No treatment-related findings were reported; however, no specific statement was given regarding ophthalmological results and no individual or summary data are in the report.

E. BLOOD WORK1. Hematology

Dietary exposure to Thiophanate-methyl resulted in small changes at 18 months in two hematological parameters that were statistically significant ($p < 0.05$ or 0.01) by the study author's analysis, but these were not dose-related or toxicologically meaningful. These included a transient 14% decrease in hematocrit in the 10 ppm female group, a 16% increase in RBC count in the 40 ppm males and a 14% increase in RBC count in 640 ppm females. At 24 months, slight decreases in hematocrit in females and RBC count in both sexes (not significant according to

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the study authors) were within 10% of control values. Overall, the hematological results were considered to be within normal ranges and not different from the control values. The required assays for hemoglobin, platelet count, and a blood clotting measurement were not conducted.

2. Clinical chemistry

There were no apparent treatment-related effects on clinical chemistry parameters. At 12 months' treatment, one rat of each sex in the control and most treatment groups had high (>50 King-Armstrong units) serum alkaline phosphatase activity but no other clinical chemistry correlates of abnormal liver function, no organ weight changes, nor histopathological correlates. These values are thus not thought to be toxicologically meaningful or related to treatment. Protein-bound iodine values generally decreased with age in both controls and treated groups, but no treatment-related effects were apparent. Other measured clinical chemistry indices were normal at all observed time points. Increased BUN in the 640 ppm females at 12 months (225% above controls) was due to an outlier. No required electrolyte or mineral levels were measured, nor were the required enzyme, serum alanine amino-transferase (SGPT), or blood creatinine.

F. URINALYSIS

By 18 months, age-related increases occurred in urine protein values in both control and treatment groups in association with other evidence of nephropathy; no treatment effects were apparent. No abnormal results were observed in any other urinalysis endpoints.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

No significant changes in any organ weight were seen in the animals sacrificed after 58 weeks' treatment with Thiophanate-methyl except for some kidney changes. A few kidneys from animals in all groups showed mild increases in the kidney-to-body weight ratio. Two rats (animal numbers unspecified) were stated to have a much higher degree of hypertrophy, together with cardiomegaly and high BUN, which were possibly associated with renal hypertension. These renal changes were not treatment-related.

At terminal sacrifice (103 weeks), no treatment-related, biologically important changes were observed in group mean absolute organ weights or organ-to-body weight percentages. No organ-to-brain weight ratios were calculated by the study author. A few group mean organ weight differences were identified by the study author as statistically significant from control values either in absolute terms or relative to body weight; most were scattered, inconsistent and not dose-related. At 640 ppm, absolute heart weight was reportedly significantly decreased in males at 24 months (15% less than controls) and liver weight in females at 12 months

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(19%), but this appeared to be related to decreased body weight since no significant differences were observed in relative weights. There were no effects on heart weight in females. Decreased absolute/relative lung weights in 640 ppm males (30%/20% less than controls, abs/rel) at 24 months were also observed but were not associated with any pathology. These differences are judged not to be toxicologically significant.

2. Gross pathology

Testicular atrophy was reported to occur in a dose-related pattern at necropsy (see Table 4, below) in animals exposed more than 58 weeks. Testicular atrophy was also observed in some animals dying on test after 58 weeks' exposure; together with those sacrificed at 103 weeks. The increase in total incidence of testicular atrophy is statistically significant ($p < 0.05$) in the high-dose rats compared to controls; it is statistically significant at 160 ppm as well as 640 ppm ($p < 0.05$) if those rats having pituitary abnormalities are eliminated. However, the toxicological significance of the increased incidence of grossly visible testicular atrophy is unclear because there was not a good correlation with the microscopic lesions that were observed in the testes (see below), although the testes weight data that was available for a few of the affected animals did show decreased weights. In addition, some testicular atrophy would be expected in 2-year old rats. The most frequent gross observations, seen in both control and treatment groups, were chronic kidney and lung inflammation and tumors. The tumors were mostly benign, and occurrence was scattered in all groups with no evidence of dose response. Other macroscopic findings also do not appear to be related to test material administration.

TABLE 4. Grossly visible testicular atrophy in rats given Thiophanate-methyl for 24 months					
Incidence	Dose (ppm)				
	0	10	40	160	640
Testicular atrophy, total incidence ^{1,2}	1/39 (2.5) ³	1/21 (5.0)	2/22 (10)	4/24 (17)	5/25* (20)
Testicular atrophy, without pituitary tumor or cyst	0/38	0	1/22 (4.5)	4/24* (17)	3/23* (13)

Data taken from Tables 1-1 to 1-18, pp. 33-50, and Tables 16-1 to 16-40, pp. 127-166, MRID 00117868.

Statistically significant by Fisher's exact test, * $p < 0.05$; calculated by reviewer.

¹Positive for treatment related trend by the Cochran-Armitage trend test, performed by reviewer.

²Number of animals in group reduced (by reviewer) by those found dead in a state of advanced autolysis that precluded histopathological examination and by those in interim sacrifice groups or animals sacrificed prior to the interim sacrifice.

³Numbers in parentheses are percent showing effect, calculated by reviewer.

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3. Microscopic pathologya. Nonneoplastic

Two types of apparently treatment-related changes were observed [see Table 5, below; note that this table includes rats from intermediate studies (14 and 58 weeks), rats exposed for 103 weeks, and rats dying at intermediate times]. At 640 ppm, mild thyroid hypertrophy (increase in height of follicular epithelium and in colloidal substance) was reported in male rats, with increases in both incidence and severity in the high-dose group (17% incidence, calculated by reviewer) over controls (2% incidence) and two lower dose groups (6% and 8.6% incidence, respectively). There were no clear increases observed in the 14- and 58-week scheduled sacrifice animals. (It is noted that there is a discrepancy between the individual animal data for control males in the interim and final reports: the former lists 1 control male as having thyroid hypertrophy in the 12-month group, whereas the latter lists none for that period). Table 5, below shows results taken from the final report). The second type of treatment-related effect was an apparent dose-dependent effect on the incidence of spermatogenesis suppression. The incidence of spermatogenesis suppression in the high-dose group was 24% compared to 2.5% in the control group and 4 to 8% in the intermediate dose groups using numbers from Table 14-2, MRID 00117868 (percent increases calculated by the reviewer). No decreased spermatogenesis was observed in the interim sacrifice groups. The increased incidence of such changes in the 640-ppm group was statistically significant ($p \leq 0.05$) by the Fisher exact test (calculated by reviewer) when animals and their controls sacrificed at 103 weeks and those dying on test after 58 weeks were considered. One male at 40 ppm showed microscopically visible testicular atrophy but this was not observed at other dose levels. Various spontaneous lesions including renal changes associated with all stages of chronic progressive nephropathy, lung inflammation (pneumonia) and other incidental findings were seen but are not considered to be treatment-related. They were of the expected type and severity for rats of this age and strain. No treatment-related microscopic lesions were reported in females.

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TABLE 5: Incidence and severity of microscopic changes found in thyroids and testes of rats given Thiophanate-methyl for 24 months					
Endpoint incidence, severity	Dose (ppm)				
	0.00	10	40	160	640
Males					
Thyroid hypertrophy, total incidence ¹ (all animals on study)	1/50 (2) ²	2/34 ³	3/35 (9)	0/34	6/35 (17)
Grade 1	0/50	2/34	3/35	0/34	3/35
Grade 2	1/50	0/34	0/35	0/34	3/35
Incidence in 14 and 58-week sacrifice groups, Grade 1	0/10	1/10	1/10	0/10	2/10
Spermatogenesis depression in terminal sacrifice groups only	1/40	1/24	1/25	2/24 (8)	6/25*(24)
1TS	0/40	0/24	0/25	0/24	1/25
2TS	1/40	0/24	0/25	2/24	5/25
3TS	0/40	1/24	1/25	0/24	0/25
Incidence in 14 and 58-week sacrifice groups	0/10	0/10	0/10	0/10	0/10
Total incidence, all animals on study	1/50	2/34	1/35	2/34	6/35 (17)
Spermatogenesis depression, all dying or sacrificed after 58 weeks ^{7,8}	0/38	0/21	1/22	1/24	6/23** (26)
Females					
Thyroid hypertrophy ¹ , total incidence (All animals on study)	2/46	1/33	1/35	0/35	2/35
Grade 1	1/46	1/33	0/35	0/35	2/35
Grade 2	1/46	0/33	1/35	0/35	0/35

Data taken from Tables 16-1 to 16-40, pp. 127-166, MRID 00117868.

Statistically significant by Fisher's exact test, *p < 0.05; **p < 0.01, calculated by reviewer.

¹Thyroid hypertrophy: height of colloidal epithelium increased.

²Numbers in parentheses are percent showing effect, calculated by reviewer.

³Number of animals in group reduced (by reviewer) by those dying in a state of advanced autolysis that precluded histopathological examination.

⁴1TS: Diminution of spermatogenesis.

⁵2TS: Hypospermatogenesis.

⁶3TS: Aspermatogenesis.

⁷Excluding animals with pituitary tumors or one with cyst.

⁸Positive for trend by Cochran-Armitage trend test, conducted by reviewer.

b. Neoplastic

There were no treatment-related neoplastic findings. No quantitative incidence data were presented. Tumors, most of which were benign, were seen at 58 and 103 weeks' sacrifice in both control and treated animals. These were

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not treatment-related lesions. They included mainly mammary fibroadenomas and pituitary tumors (chromophobe adenomas). The tumor latency was not treatment-related. Other tumor types seen in the 24-month study included adrenal cortical and medullary adenomas, ovarian cystomas, pancreatic adenoma, pulmonary adenomas, lung fibrosarcoma, renal cortical adenoma, pancreas and liver histiocytomas, leukemia, thyroid adenoma, liver adenoma and parathyroid adenoma.

III. DISCUSSION

A. DISCUSSION

The reviewer agreed with the conclusions of the study authors. The major target organs in this study were thyroid and testes in males. In addition, decreases in body weight/weight gain were observed in both sexes.

Mild decreases were seen in the high-dose male rat body weights ($\leq 13\%$) and body weight gains (4.0% at 12 months, 16% at 24 months). Corresponding decrements were observed in high-dose female rat body weights (16% at 24 months) and body weight gains (24% at 12 months, 19% at 24 months). Statistical analysis by ANOVA (by reviewer) showed no statistically significant decreases in final body weights or overall body weight gains in either sex. The study author had claimed a statistically significant decrement in overall body weight gain in both sexes at 24 months, by an unspecified statistical test (replication of their result is obtained by the paired t-test). The decreases are considered treatment-related due to their magnitude ($>10\%$) and occurrence in both sexes. Slight decreases in food consumption in high dose females ($\leq 12\%$) suggest a possible palatability problem, although overall food efficiency as estimated by the reviewer was also decreased (by 11% for males and 9.4% for females). The toxicological significance of these decrements is uncertain, especially since weekly food efficiency data are not available to evaluate temporal variations.

In the gross examinations, testicular atrophy was increased in a dose-dependent pattern in animals sacrificed after week 58, even when animals with pituitary abnormalities (tumors, cyst) are excluded. However, the grossly visible atrophy showed no correlation with microscopic effects. There were no effects on mean testicular weights, although in the few animals with atrophy where testes were weighed, weights were decreased relative to other animals in the group. Furthermore, some testicular atrophy would be expected in older male rats. No other treatment-related effects on gross pathology endpoints were reported.

Two apparently treatment-related effects were observed in males upon microscopic examination. One was an increased incidence of thyroid hypertrophy (increased height of thyroid epithelial cells and increased colloidal substance) in the high-dose male group which was significant by the Fisher exact test when only animals sacrificed at 103 weeks and the corresponding controls were considered, although it was not accompanied by increased thyroid weight or thyroid tumor formation. In the other case, the incidence of microscopically-determined spermatogenesis suppression is also

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statistically significant by the Fisher exact test ($p < 0.05$) when only high-dose animals and their controls sacrificed at 103 weeks or dying after 58 weeks are evaluated.

The study author indicates that in a separate study using Thiophanate-ethyl, similar thyroid and testicular effects were observed, lending credence to these as real effects in this study. The 1993 two-year chronic feeding/oncogenicity study (MRID 42896601) in a different rat strain (Fischer 344) showed that Thiophanate-methyl had similar effects on the thyroid but at higher dose levels. More pronounced thyroid effects than in the present study were seen in both males and females at 1200 ppm (54.4 mg/kg/day in males, 63.5 mg/kg/day for females) and above, but none at 200 ppm (8.8 mg/kg/day for males, 10.2 mg/kg/day for females). Toxicologically significant effects on body weight were observed only at 1200 and 6000 ppm in males and at 6000 ppm in females. No testicular effects were reported in this study.

Other studies received by the Agency, including a recent Sprague-Dawley rat 2-generation reproductive toxicity study (MRID 4289901-05, 43624401; review in HED doc. No. 011748) also showed no testicular effects. At this time it is not known whether the decreased spermatogenesis observed in this chronic study reflect different strain sensitivities or some other factor. In a published report (MRID 00081605)¹, the effects of thiophanate and thiophanate-methyl on testes were investigated in male Swiss-Webster mice. In that study, 8 male mice/group were administered 295 mg/kg/day of thiophanate or 192 mg/kg/day of thiophanate methyl or vehicle only (1% Methocel) for 5 consecutive days. Doses selected were 1/50 and 1/18 the LD₅₀ for thiophanate and thiophanate-methyl, respectively and were limited by the small amount of sample made available for testing. Animals were then evaluated for effects on spermatogenesis, assimilation of testosterone in the prostate and weights of testes, seminal vesicles, prostate and adrenal glands. The only treatment-related findings were increases in prostate:body weight ratios for thiophanate-methyl (26% above untreated mice) and increased adrenal:body weight ratios for thiophanate (47%) and thiophanate-methyl (94%). The effects of longer-term compound administration or different dose levels were not evaluated.

No treatment-related tumors were reported; however, there were relatively small numbers of surviving animals in some groups.

B. STUDY DEFICIENCIES

No statistical analysis was conducted by the study author in assessing the histopathology incidence results. Even those animals which died and on which no histopathological analysis was possible due to advanced autolysis were included in histopathology summary Tables 14-1 and 14-2 (MRID 00117868), although these represented only a few animals.

¹Thomas, J.A. and Schein, L. (1974) "Effects of Thiophanate and Thiophanate-Methyl on the Male Reproductive System of the Mouse". *Toxicol. and Appl. Pharmacol.* 30:129-133.

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The method(s) of statistical analysis of body weight data was not identified, but a replication of the results for overall body weight gain suggests that a paired t-test was used. The reviewer's analysis by one-way ANOVA showed no statistically significant effects on final body weight or overall body weight gain in either male or female groups. However, these decreases were still considered treatment-related.

No individual animal body weight data except for the initial and final or terminal body weights were provided or individual or cage food consumption data. These required data were reportedly collected but are not presented in the report.

Analytical verification of test material homogeneity, stability and actual concentration in the diet mixtures or purity of Thiophanate-methyl were not given in the report.

Food consumption was determined not weekly but fortnightly. Data are not reported at every interval or for individual rats or cages, but only summarized as cumulative consumption at 3, 6, 13, 18, and 24 months. This means that test compound consumption estimates and corresponding dose estimates are confounded.

Age was not given; rats may not have been of uniform age or all in good health, judging from initial weight data and the fact that two animals died the first week on test from lung infections. The animals do not appear to have been distributed randomly by weight across dose and control groups; differences between initial group mean weights are slightly >20% in some cases. This deficiency does not appear to have affected the interpretation of body weight effects, as the initial mean body weights for high-dose groups were close to those of the control groups.

Results of ophthalmoscopic examinations were not included. These were listed as being performed on all animals at 0, 3, 6, 12, 18, and 24 months (MRID 00057651).

Other deficiencies include omission from the hematology assays of required tests including hemoglobin determination, platelet counts, a measure of clotting time, and clinical chemistry assays of all electrolytes, blood creatinine, and the required enzyme, serum alanine amino-transferase (or SGPT).

Several required tissues were not preserved for microscopic examination (esophagus, cecum, rectum, trachea, and peripheral nerve). The report does not clearly state whether all gross lesions and masses were preserved and examined; the discussion and presence of photomicrographs of a few lesions suggests that most were examined. It is not clearly stated whether most of all other organs/tissues preserved were examined; nearly all were examined from the intermediate sacrifice in all dose groups and it appears from the summary tables (14-1 and 14-2, pp. 124-5, MRID 00117868) that this is the case with the terminal sacrifice rats. Although thymus weights were given in the interim study report and were stated to have been collected at termination, no data were provided in the final report. The tables of individual rat histopathology results do not list aorta, bone, or skeletal muscle as being examined at 24 months. However, they show blood and "subcutaneous" which are not listed in the methods. This inconsistency does not appear likely to change the interpretation of results.